NOTES ON OSMOTIC EXPERIMENTS WITH MARINE ALGAE[1]

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During the summer of 1899, when the writer was engaged in plant physiological investigations at Woods Hole, he took the opportunity to study the osmotic properties of a number of algae from both fresh and marine waters. These studies were not complete, but since they shed some light on relations which still have much physiological interest, the results are here presented. Moreover, since that time, through the work of MORSE and his associates (15), BERKELEY and HARTLEY (1, 2, 3), and others, the recalculations of osmotic relationships have resulted in important changes. The bearing of the work of physicists on the problems of physiology has been pointed out by RENNER (20), who has done much to resolve the difficulties involved in the question. The osmotic values here dealt with have been calculated according to the newer methods. In some cases the values calculated according to PFEFFER'S (18) data are added in order to enable the reader to contrast the values obtained under the two methods of reckoning.

Osmotic pressure in Spirogyra cells

It was desired first to ascertain an approximate measure of the osmotic value of the sea water at Woods Hole. For this purpose some organism having a lower osmotic pressure than that of the sea water was sought. Several fresh water algae, *Spirogyra elongata*(?), *Zygnema* (sp.), and *Oedogonium* (sp.), found growing in a small fresh water pond between Woods Hole and Nobska Point, were tested.

Preliminary experiments with the distilled water available showed the presence of injurious impurities, probably copper from the still. The addition of shredded filter paper to the stock bottle was found to remove the pathological symptoms, and the solutions

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used were made up with distilled water so treated. In all cases solutions were made up on the basis of the desired number of gram molecules of dry substance dissolved in water sufficient to make a liter of *solution*, that is, on the volume-normal basis.

In order to calibrate the indicator plants, solutions of cane sugar and of NaCl in a graduated series of concentrations were carefully prepared from high grade chemicals. These dilutions when in use were kept in covered beakers of 250 cc. capacity. The algae were quickly freed of surplus solution by the use of fresh filter paper before transfer, and freed from remaining traces of the solution by a quick rinsing in a duplicate portion of the solution into which they were to go. After the transfer, filaments were removed at definite intervals for microscopic examination, either on a watch glass or on a slide.

In determining the osmotic equivalent, some difficulty was experienced owing to the fact that all cells of the same filament did not show the same plasmolytic response to a given concentration. This difference was especially marked as the critical concentration was approached. As a rule the tip cells of a filament showed incipient plasmolysis in a weaker solution than did the other cells. Those that had lately undergone division seemed to plasmolyze more promptly as a rule. Since plasmolysis begins to take place only after the concentration of the outer medium is in excess of the concentration of the cell sap, in this study the osmotic end reaction was regarded as reached when the first traces of withdrawal of the protoplast were seen in the tip cells. Since the problem of absorption was not under investigation, the persistence of signs of plasmolysis was not studied. In order to avoid as far as possible complications due to the penetration of the materials from the solution under test, results seen within an hour after the application of the solutions in question were accepted. At times slight plasmolysis seen within this time would soon disappear. Obviously, therefore, the promptest possible registration of osmotic conditions would be expected to give the best evidence of conditions normally existing in the cell.

As a result of a series of tests made with cane sugar, it appeared that for a major part of normal *Spirogyra* and *Oedogonium* cells a concentration of 0.25 gm. molecules per liter of solution was just short of producing plasmolysis at 22°0 C. Only in the tip cells was

an undoubted "starting" of the protoplast from the wall seen. This appeared inside of 20 minutes and still persisted at the end of an hour, but was scarcely noticeable after 20 hours. The osmotic pressure of the cell contents of these algae was, therefore, very nearly equal to 0.25 gm. mol. of cane sugar in a liter of solution. In calculating this value in terms of atmospheres, the values of MORSE and FRAZER (**15**) were used. However, since MORSE'S osmotic determinations were made on the basis of gram molecules dissolved in 1000 gm. of water, this value was reduced to the latter basis by means of RENNER'S formula: $\frac{m \quad 1000}{1000-214m}$, m being the given number of gram molecules in 1000 ccm. of solution. The osmotic equivalent of the algae in question became, therefore, 0.264 gm. mol. in 1000 gm. water. The osmotic values of a series of cane sugar solutions determined in atmospheres by MORSE (**16**) and associates were plotted in a series of curves on which by interpolation the osmotic value of 0.264 gm. mol. weight-normal at 22° C. (the temperature at which the plasmolyzing solutions stood at the time of the determination) was found to be about 6.7 atmospheres. According to PFEFFER the corresponding value would be about 5.9 atmospheres.

Tests on *Spirogyra* showed that the cell contents were osmotically equal to a solution of NaCl containing about 0.16 gm. mol. in a liter of solution. In solutions of NaCl of this degree of dilution the difference between volume-normal and weight-normal is negligible in view of the wide range of error in the biological data. This may be seen by calculating weight-normality in accordance with the following formula given by RENNER (p. 500). When M equals the molecular weight of NaCl (58.5), m equals the number of gm. mol. per liter of solution, and s the specific gravity of the given solution, the corresponding weight-normality equals $\frac{1000m}{1000s-m.M}$. The specific gravity (20°/4° C.) of a 0.16 volume-normal NaCl (0.93 per cent) solution obtained by interpolation on a curve based on LANDOLT-BÖRNSTEIN-ROTH (**13**, p. 260) is about 1.005. Solving the equation, the corresponding value weight-normal is 0.1607 gm. mol. in 1000 gm. water.

The osmotic value of 0.16 gm. mol. NaCl in terms of atmospheres is not so readily deducible in this case as in that of cane sugar, and in view of the physical difficulties discussed by RENNER the writer has taken the corrected osmotic values given by him (p. 501) as a basis of calculation. By interpolation the osmotic pressure of 0.16 gm. mol. NaCl is about 7.2 atmospheres at room temperature. According to PFEFFER this corresponding value would be 5.7 atmospheres.

In this concentration the cell contents became markedly disordered after a short time, the chlorophyll band largely losing its spiral form. However, tests with solutions of cane sugar, slightly stronger osmotically, showed prompt and apparently normal plasmolysis. After 24 hours in this solution the chlorophyll band was still further disordered, although nearly all cells were clearly living and plasmolyzed normally in stronger concentrations.

Osmotic value of sea water

By the use of cane sugar solutions the osmotic pressure of *Spirogyra* here used was found to be about 6.7 atmospheres; the use of NaCl solutions gave about 7.2 atmospheres. Since the difference between these values is without doubt exceeded by the differences in the osmotic pressures prevailing in individual cells of the same filament, there is perhaps little point in discussing which of these values shall be adopted as the basis of further calculations. Hence, an approximate value of 7.0 atmospheres is adopted as the basis of further discussion.

The sea water used was dipped from outside the Fish Commission pier, where it is subject to almost unceasing tidal movement, and gave a density reading of about 1.0210 at 71° F. This was diluted with distilled water in various proportions and used as a plasmolyzing agent for *Spirogyra*. A stage similar to that just noted as indicating beginning plasmolysis was seen in a mixture containing *30 parts* by volume of sea water to *70 parts* of distilled water at 22° C. In this concentration *Spirogyra* and *Oedogonium* agreed in showing faint indications of incipient plasmolysis. *Mesocarpus* showed more distinct traces. These traces disappeared inside of 24 hours.

It appeared from these experiments that the osmotic presure of a 30 per cent sea water solution was approximately equal to about 7.0 atmospheres. By plain calculation the osmotic value of undiluted sea water would be about 23.3 atmospheres.

Since, however, it is well known that salts in aqueous solutions dissociate electrolytically in greater proportion in dilute solution than in greater concentrations, a given number of molecules might through their ionization be expected to cause a proportionally greater osmotic pressure at 30 per cent dilution than in a solution having three times that concentration. In order to get an idea of the general order of magnitude of the change here concerned, it is assumed that the behavior of the sea water approximates that of a half-normal NaCl solution. In this solution, corresponding to the undiluted sea water, about 73 per cent of the molecules would be dissociated at 18° C. (KOHLRAUSCH and HOLBORN, **12**), while a 30 per cent sea water solution corresponding roughly to N/6 concentration of NaCl would be dissociated about 81 per cent. This would increase the relative osmotic value from 173 to 181. This difference amounts to about 5 per cent of the osmotic value of the N/2 solution. To correct for this overestimate would require the subtraction of about 1.0 atmosphere from the first calculation. This would give an osmotic value of about 22.3 atmospheres for the sample of sea water here used.

In this connection it is of interest to compare this approximation with other determinations of this value. The salt content of the sample of sea water used may be calculated from the specific gravity reading 1.0210 at 71° F. This reading, reduced to a basis of specific gravity $\frac{15^\circ C.}{4^\circ C.}$ by means of LIBBEY'S (**14**) table, becomes 1.0216. This value reduced to terms of salt content by the use of PETTERSON'S (**17**) comparison of specific gravity with results obtained by titration of Cl content indicates a total salt content of about 2.93 per cent.[2] Assuming this result to have been approximately correct, it is possible by use of the "Challenger" (**7**) analyses to ascertain

[2] A discussion of the methods of calculating specific gravity and salt content with a diagram for the ready handling of these data is found in Science N.S. 42:732–735. 1915.

the quantity of principal salts present, and by means of their osmotic equivalents to calculate roughly the osmotic value of the sample of sea water used in this work.

PFEFFER has calculated the osmotic equivalents of solutions of the common salts, giving the atmospheres of pressure exerted by 1 per cent solutions made up on the basis of 1 gm. of salt in 100 ccm. of solution. The recalculation of the osmotic value of NaCl by RENNER already referred to has given a considerably increased value for this salt. There has been no similar recalculation for the other sea salts known to the writer, but since the quantities of salts other than NaCl are small, but a relatively small effect would result from their correction. $MgCl_2$, present in second largest quantity, namely, 0.32 per cent in the sample of water here concerned, was recalculated by the writer in a very approximate way from freezing point values given in LANDOLT-BÖRNSTEIN-ROTH'S (13) tables for the temperature of 22° C., and a value somewhat greater than that given by PFEFFER was obtained. The values here discussed are brought together for convenient reference in table I. A glance at

TABLE I

Salts	"Challenger" proportions	Quantity in sample used	Atmos. press. 1 per cent sol. by vol. Pfeffer	Osmotic values Pfeffer	Osmotic values recalculated
$NaCl$....	0.777×2.93 =	2.28 per cent	×6.09 atmos.	= 13.8 atmos.	17.30 atmos.
$MgCl_2$....	0.109×2.93 =	0.32	×4.98	= 1.6	2.16
$MgSO_4$...	0.048×2.93 =	0.14	×1.93	= 0.3	0.30
$CaSO_4$....	0.036×2.93 =	0.10	×2.00(?)	= 0.2	0.20
K_2SO_4....	0.025×2.93 =	0.07	×2.72	= 0.2	0.20
	0.995	2.90 per cent	equals	16.1 atmos.	20.16 atmos.

this table shows that if PFEFFER'S osmotic values are accepted, the osmotic pressure of sea water falls short of that contained in the experiment described by a ratio of 16.1 to 22.3. On recalculating, the total pressure derived from analytical data exceeds 20 atmospheres.

In this connection it is of interest to compare with these values those obtained by GARREY (10), using the freezing point method. As a result of several freezings, he concluded that for the water of the basin of the United States Fish Commission the average lower-

ing of the freezing point was −1.82° C., corresponding to an estimated osmotic value of about 22 atmospheres at 0° C. (about 23.7 atmospheres at 22° C.). Assuming the osmotic value of a 1 per cent NaCl solution at 22° C. to be 7.6 atmospheres, in accordance with RENNER'S recalculation (p. 501), GARREY'S result would call for a salt content equal to about 3.1 per cent NaCl. According to SUMNER, OSBURN, and COLE (**22**), the water of Buzzard's Bay and Vineyard Sound varies in salt content between 2.84 and 3.29 per cent total salt.

Osmotic pressure of marine algae

An attempt was made to determine the osmotic pressure existing in certain of the commoner bright green forms found abundantly in the neighborhood of Woods Hole.

Cladophora gracilis var. was found growing on rocks below low tide level near the wall in front of the residence building of the United States Fish Commission. This alga grew in a position where the water was constantly changing and where it was not subject to any marked temperature variation.

Enteromorpha intestinalis, according to DAVIS (**6**), is a type belonging characteristically to the region between tide levels, where it occurs attached to stones, shells, and woodwork. At low tide, therefore, it is often subject to a considerable concentration of its cell contents through evaporation.

Chaetomorpha Linum, like *Cladophora*, is not subject to such wide variations, being found characteristically below the low tide mark.

Small tufts of the filaments or pieces of the frond were placed in graduated series of solutions of cane sugar and NaCl and examined with reference to their osmotic behavior.

In the cane sugar solutions *Cladophora* first showed traces of plasmolysis in 0.85 to 0.90 gm. mol. per liter of solution, corresponding to 1.04 and 1.13 gm. mol. in 1000 gm. of water, corresponding to about 28 and 30.7 atmospheres of pressure respectively.

Enteromorpha gave similar results in solutions containing between 0.80 and 0.90 gm. mol. volume-normal, corresponding to about 0.96 and 1.13 gm. mol. weight-normal, representing 25.8 to

30.7 atmospheres, respectively. *Chaetomorpha* required a 0.9 volume-normal concentration (1.13 weight-normal), corresponding to 30.7 atmospheres, to produce the same effect.

In NaCl solutions corresponding results were seen in *Cladophora* in 0.75 to 0.80 gm. mol. volume-normal. RENNER (p. 501) has pointed out that in NaCl the osmotic pressure is proportional to the molar concentration calculated on the liter of solution, 0.1 gm. mol. having an osmotic pressure of 4.5 atmospheres at 18° C. Hence these concentrations correspond approximately to a range between 33.7 and 36 atmospheres. *Chaetomorpha* showed first traces of plasmolysis in 0.70–0.80 gm. mol., corresponding to 31.5–36 atmospheres of pressure.

The reason why an osmotically greater concentration was required in the case of the NaCl solution to give the same result as that seen in the osmotically less concentrated sugar solution is probably to be found in the greater facility with which these algae admit NaCl. It is probable that the surplus atmospheres required in the NaCl solution over the sugar solution roughly mark the greater degree of penetration of the former. The work of JANSE (11) and of DREVS (8) is significant in this connection.

In 1900 and 1901 DUGGAR (9) carried out similar plasmolytic studies on marine algae at Naples and at Woods Hole. The results presented in his paper seem to have been obtained at Naples, since the values are referred to Naples water. Experiments on *Chaetomorpha Linum* made with solutions of osmotic agents in distilled water, as would be expected, showed markedly higher osmotic pressures than the writer found at Woods Hole. At Naples *Chaetomorpha* was found to be isosmotic with 1.26 gm. mol. by volume cane sugar or 1.73 gm. mol. by weight, having an osmotic pressure of about 34.7 atmospheres; with 0.93 gm. mol. of NaCl by volume equal to about 41.8 atmospheres; and with 1.40 gm. mol. by volume of KNO_3. The freezing point of Mediterranean water was found by BOTTAZZI (4) to be $\Delta = -2.29°$ C., corresponding to about 27.6 atmospheres at 0° C., or about 30 atmospheres at 22° C. This is the equivalent of nearly 1 per cent NaCl, or 6.2 atmospheres higher than values obtained at Woods Hole by GARREY. Analyses of Mediterranean water from Naples reported by ROTH (21) gave

a total salt content of about 3.85 per cent, a value which agrees very well with these findings.

In 1901 REED (19) made a series of plasmolytic determinations with marine algae at Woods Hole and in solutions made up with distilled water found the following osmotic values: *Cladophora* (sp. not given): NaCl isosmotic with 0.7 gm. mol. NaCl by volume, roughly equal to 31.5 atmospheres at 18° C.; cane sugar isosmotic with 0.8 gm. mol. by volume (0.965 gm. mol. by weight), equal to 25 atmospheres of pressure at 18° C. *Chaetomorpha* (sp. not given): NaCl isosmotic with 0.9 gm. mol. by volume, equal to about 40.5 atmospheres at 18° C.; cane sugar isosmotic with 0.9 gm. mol. by volume (1.11 gm. mol. by weight), equal to about 29.5 atmospheres at 18° C.

Osmotic surplus in marine algae

In order to ascertain the turgor pressure of the marine algae, a comparison between the osmotic value of the cells and that of the sea water itself is necessary. To facilitate such a comparison these values are brought together in table II. For such a calculation as

TABLE II

OSMOTIC PRESSURES OF ALGAE AT WOODS HOLE

ALGAE	CELL CONTENTS ISOSMOTIC WITH				
	Cane sugar gm. mol. in liter of solution		Sodium chloride gm. mol. in liter of solution		Sea water per cent by volume
	gm. mol.	atmos.	gm. mol.	atmos.	
Spirogyra elongata	0.25	6.7	0.16	7.2	30 Osmotic pressure of sea water corrected = 22.3 atmos.
Cladophora gracilis	0.85 0.90	28.0 30.7	0.75 0.80	33.7 36.0	
Enteromorpha intestinalis	0.80 0.90	25.8 30.7	....	....	
Chaetomorpha Linum	0.9	30.7	0.70 0.80	31.5 36.0	

that here required it is important to adopt a correct osmotic value for sea water. For purposes of this paper 22.3 atmospheres, corresponding to a sea water concentration of 2.93 per cent total salt, is adopted. A glance at the daily density readings made by the United States Fish Commission shows a considerable variation in the salt content of Woods Hole water from time to time, a fact that

should be borne in mind in comparing the results of different observers. It seems from the observations of SUMNER (**22**, p. 53) and his associates that the salt content at Woods Hole is known to vary between 2.84 and 3.29 per cent total salts.

The osmotic surplus found in the algae studied is easily calculated by subtracting 22.6 atmospheres from the observed osmotic pressures. The results of such a calculation appear in table III.

TABLE III

OSMOTIC SURPLUS IN MARINE ALGAE AT WOODS HOLE

ALGAE	OSMOTIC SURPLUS DETERMINED WITH	
	Cane sugar	Sodium chloride
Cladophora gracilis	5.4 atmospheres 8.1	11.1 atmospheres 13.4
Enteromorpha intestinalis	3.2 8.1	....
Chaetomorpha Linum	8.1	8.9 13.4
Average values	6.6 atmospheres	11.7 atmospheres

The strikingly higher values obtained with NaCl are probably due to the penetration of this substance with the consequently higher concentration required to produce traces of plasmolysis. The writer, therefore, is inclined to regard the lower reading obtained with cane sugar as more nearly the true value in this case. It should be borne in mind, however, as COPELAND (**5**) has shown, that this osmotic surplus is subject to influence from external conditions through their effect on nutrition and in other ways.

Summary

1. By means of the plasmolytic method it is shown that the osmotic pressure in the cells of *Spirogyra*, *Zygnema*, and *Oedogonium* found in Nobska Pond, near Woods Hole, Massachusetts, at 22° C., is equal (1) to about 0.25 gm. mol. in a liter of solution of cane sugar, corresponding to 6.7 atmospheres, (2) to about 0.16 gm. mol. NaCl per liter of solution, corresponding to 7.2 atmospheres, and to a 30 per cent sea water solution (sea water = 2.93 per cent total salts).

2. The osmotic value of the sea water sample calculated from plasmolytic experiments was found to be about 22.6 atmospheres. This value determined by the freezing point method by GARREY reduced to 22° C. was 23.8 atmospheres.

3. The osmotic surplus of *Cladophora gracilis*, *Enteromorpha intestinalis*, and *Chaetomorpha Linum* was found to be about 6.6 atmospheres when determined by means of cane sugar, and 11.7 atmospheres for *Cladophora* and *Chaetomorpha* when determined by means of NaCl. The penetration of NaCl is supposed to be largely responsible for the higher value obtained with this salt.

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